
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to prevention / therapy agent and the osteoclasis inhibitor of absorptivity bone diseases, such as a hypercalcemia by osteoporosis, a malignant tumor, etc. which are characterized by making activated protein C (it abbreviating to APC hereafter), and/or pro TIN C (it abbreviating to PC hereafter) into an active principle, or a Paget's disease of bone.
[0002]
[Description of the Prior Art] The osseous tissue is continuing metabolic turnover continuously, a part of existing bone is absorbed by the osteoclast, and, on the other hand, the new bone is formed of osteoblast. A bony gestalt and bone quantity are maintained by this bone turnover. Bone quantity is determined by functional total of the osteoclast group which absorbs a bone, and the osteoblast group which forms a bone (2267 2262- Hideki Yoshikawa et al., a Japanese clinical one, 52, 1994).
[0003] An absorptivity bone disease is a disease which the amount of osteoclasis by the osteoclast group serves as size from the amount of osteogenesis by the osteoblast group, and reduction in bone quantity produces as a result. There is a Paget's disease of bone by osteoporosis, the malignant hypercalcemia which myeloma and a lymphoma start owing to, and locality osteoclasis in an absorptivity bone disease. There are involution osteoporosis and secondary osteoporosis as osteoporosis, and retrogressive osteoporosis has in it the I-beam osteoporosis accepted after a menopause or an ovariectomy, and II mold osteoporosis which an old man is permitted further. Secondary osteoporosis is osteoporosis with a clear cause, and chronic articular rheumatism, steroid hormone therapy, hyperthyroidism, hypogonadism, hyperparathyroidism, acromegaly, diabetes mellitus, Cushing's syndrome, Turner's syndrome, administration of an immunosuppresant, gastric resection, renal failure, kidney dialysis, an osteogenesis imperfecta, low alkaline phosphatase ****, a homocystinuria, nutritional disorder, limitation of movement, bedridden, alcoholism, a space flight, etc. become a cause. Moreover, the idiopathic juvenile osteoporosis which is not clear also has a cause. In addition to the height of the frequency, an absorptivity bone disease causes fracture of each part of the body, and attracts attention also not only as current and medical care with which an aging society progresses but as a social problem as a cause disease in which the so-called "bedridden elderly" is made to result.
[0004] The osteoclast group and the osteoblast group were in charge of the manifestation of the function, and have received accommodation by humoral factors and extracellular-matrix proteins, such as various hormone and cytokine. As a factor which carries out direct inhibition of differentiation and activation of an osteoclast, calcitonin, a prostagladin, a cysteine protease inhibitor, C terminal parathyroid related protein, etc. are known, and development of absorptivity bone disease therapy agents, such as bis-phosphonate and ipriflavone, is furthered.
[0005] PC is the vitamin K dependency protein generated by liver, and is important as a controlling factor of blood coagulation. It activates with the complex of the thrombin generated in process of blood coagulation, and TRON BOMOJURIN on the blood vessel inner-bark film, and PC turns into APC. [Mahler Earl et al. who checks a coagulation cascade when produced APC carries out limited decomposition selectively and carries out deactivation of the activation V factor and activation factor VIII which are a blood coagulation factor (Marlar R.), a brad (Blood), 59, and 1067- 1072 and 1982 BEHA G A et al. (Vehar G.A.) -- BAIOKE Miss Rory (Biochemistry), 19,401-410, and 1980] -- as for PC and APC, development as anticoagulant is performed from things. Moreover, in APC, anti-inflammatory activity is also reported (Kenji Okajima et al., the 32nd Japan Society of Clinical Hematology general meeting abstract collection, WS III-10 subject, 1990), and the leucocyte activation depressant action of APC is suggested as the action mechanism. moreover, [to which it is reported that the receptor to APC is also discovered to the vascular endothelial cell -- the manifestation by osteocyte is not checked and the report which indicated having an

operation to an osseous tissue does not have FUKUDOME KE et al. (FukudomeK.), THE Journal of Biological Chemistry (The Journal of Biological Chemistry), 269, 26486-26491, and 1994], either.
[0006]
[Problem(s) to be Solved by the Invention] Until now, the calcitonin which has calcium lowering operation of a blood serum by the estrogen which is one of the female sex hormones, and promotes osteogenesis, the active vitamin D 3 which improves bone quantity reduction, the calcium preparations used as a substitution therapy, and the peptide hormone secreted from the thyroid, the elcatonin which is the derivative of calcitonin, the ipriflavone which reinforces an operation of estrogen are used for the therapy of absorptivity bone diseases, such as osteoporosis. However, in estrogen, thrombosis and the onset of cancer are reported by the metrorrhagia and chronic administration, and it considers as contraindication at the patient with the misgiving of estrogen dependency neoplasms, such as thrombosis and a breast cancer, and a uterine cancer. Moreover, side effects, such as digestive trouble, such as nausea and vomiting, a shock disease, and an anaphylaxis, are reported by an active vitamin D 3, calcitonin, elcatonin, and ipriflavone, and the effectiveness is not necessarily satisfactory. It is an important technical problem that a side effect develops a slight safe remedy also from osteoporosis being the long disease of a therapy period.
[0007]
[Means for Solving the Problem] The way and APC which are doing research on the physiological function to the various cells of APC acted on osteocyte, and this invention person found out controlling osteoclasis, and completed this invention for research in piles further wholeheartedly.
[0008] This invention is explained below at a detail. The first mode of this invention is absorptivity bone disease prevention / therapy agent characterized by containing APC and/or PC as an active principle. The Paget's disease of bone by osteoporosis, the malignant hypercalcemia which myeloma and a lymphoma start owing to, and locality osteoclasis is included in an absorptivity bone disease.
[0009] The second mode of this invention is osteoporosis prevention / therapy agent characterized by making APC and/or PC into an active principle. Retrogressive osteoporosis, secondary osteoporosis, and idiopathic juvenile osteoporosis are included in osteoporosis. Furthermore, the I-beam osteoporosis accepted after a menopause or an ovariectomy and II mold osteoporosis which an old man is permitted are included in retrogressive osteoporosis, and chronic articular rheumatism, steroid hormone therapy, hyperthyroidism, hypogonadism, hyperparathyroidism, acromegaly, diabetes mellitus, Cushing's syndrome, Turner's syndrome, administration of an immunosuppresant, gastric resection, renal failure, kidney dialysis, an osteogenesis imperfecta, low alkaline phosphatase ****, a homocystinuria, nutritional disorder, limitation of movement, bedridden, alcoholism, a space flight, etc. are mentioned as a cause of secondary osteoporosis.
[0010] The third mode of this invention is an osteoclasis inhibitor characterized by making APC and/or PC into an active principle. Usefulness is expected from the acceleration of recovery after fracture, prevention and the therapy of the fatigue fracture, or rate lifting of take of the bone grafting. Moreover, it is useful also as a reagent for research.
[0011] APC used for this invention is protein which has the property to which limited decomposition is selectively carried out and deactivation of the activation V factor and activation factor VIII in a blood coagulation cascade is carried out. Moreover, in prevention / therapy agent of this invention, the same effectiveness as APC is expected also for PC changed into APC by thrombin-TRON BOMOJURIN complex in the living body. Therefore, a natural mold or the gap produced in gene engineering is sufficient as APC and/or PC, and they may be an alteration mold obtained by the gene engineering technique. When considering as drugs, APC of the Homo sapiens origin and/or a PC are desired preferably. Furthermore, APC of the Homo sapiens origin is desired preferably. such a PC -- a natural mold -- approach [of the Kisiel W (Kisiel, W) -- PC derivative currently indicated by a journal OBU clinical investigation (Journal of Clinical Investigation), the 64,761-PC and EP No. 443875 official report which are indicated by JP,61-205487,A, JP,62-111690,A, etc. about PC manufactured by 769, 1979], etc. from human plasma and the gene recombination mold, JP,4-211380,A, etc. is raised. Moreover, although there is especially no constraint in the approach of activating PC, it can carry out by the approach (JP,3-93799,A) activated by the approach [Kisiel W (Kisiel, W) activated by the thrombin, biochemistry (Biochemistry), 16, 5824-5831, 1977], and the fixed thrombin, for example. Moreover, it can also carry out by the approach activated by snake venom or the synthetic peptide.
[0012] the protein C which is the freeze-drying article with which APC was manufactured from human plasma about the natural mold, for example, and AKUCHIBETEDDO -- human (ProteinC, Activated, Human) (SERUBIO laboratory (Serbio Laboratory) company) or pure RIFAIDO APC (PurifiedAPC) (diamond GUNOSUTIKA SUTAGO (Diagnostica Stago)) is marketed. About a gene recombination mold, activation pro TIN C currently indicated by JP,62-111690,A or the activation Homo sapiens protein C derivative currently indicated by JP,3-72877,A is raised.
[0013] It is expected that APC and/or PC will be the anticoagulant under current clinical development, there will also

be few side effects, such as a bleeding tendency which accompanies heparin, and the safety as drugs will be high. As for the Homo sapiens blood origin APC, the safety is actually checked by the single time intravenous administration toxicity test in a mouse, the general pharmacological test, virus inactivation trial, etc. (250 JP,6-183996,A, Kumiko Aoki et al., application pharmacology, 48,239- 1994). Moreover, it is known for heparin and low molecular weight heparin that bone quantity will decrease by the administration (Masahiko Nishiyama et al., the 68th Japanese Pharmacological Society annual convention abstract collection, P2293 subject, 1995). On the other hand, since APC and/or PC which are used for this invention control osteoclasis, APC and/or PC are anticoagulant which has the operation which raises bone density unlike heparin and low molecular weight heparin.

[0014] APC and/or PC which are used for this invention have osteoclasis depressant action. Therefore, it can be used as prevention / therapy agents, such as a hypercalcemia which accompanies various absorptivity bone diseases, for example, osteoporosis, Paget's diseases of bone, or malignant tumors etc. Moreover, by the collagen disease, bony rarefaction is advancing focusing on chronic articular rheumatism. Therefore, reduction of the bone quantity which is one of the osteoclasis in various collagen diseases, for example, chronic articular rheumatism, or the side effects of steroid hormone therapy can be used for prevention and the object which treats or improves, and the acceleration of a therapy of these diseases is expected further. Furthermore, since APC and/or PC which are used for this invention control osteoclasis, gathering acceleration of recovery after the fracture like each part, prevention and the therapy of the fatigue fracture, or the rate of take of the bone grafting is expected.

[0015] The pharmaceutical preparation of this invention can be manufactured according to any well-known galenical pharmacy-manufacturing methods that what is necessary is just to contain APC and/or PC as an active principle. The suitable support for which APC and/or PC concerned are generally used as drugs Or a medium, for example, sterilized water and a physiological saline, vegetable oil, mineral oil, higher alcohol, A higher fatty acid, an innocence organic solvent, etc. accept the need further. An excipient, a coloring agent, An emulsifier, suspension, a surfactant, a solubilizing agent, an adsorption inhibitor, a stabilizing agent, It combines with a preservative, a moisturizer, an antioxidant, a buffer, an isotonizing agent, an aponia-ized agent, etc. suitably. drugs pharmaceutical preparation, such as the injections and the pernasal absorbent suitable for prescribing a medicine for the patient to a living body effectively, an oral agent, and embedding pharmaceutical preparation, -- it can prepare to injections preferably and is well-known about some of these examples. As pharmaceutical preparation of injections, it can provide, for example in a freeze-drying article, the form enclosed with the water-for-injection agent or the osmotic-pressure pump. moreover -- as a sustained release drug -- a microcapsule, a microsphere, liposome, or NANOSU -- a medicine can also be prescribed for the patient as the gestalt or macromolecule basis content pharmaceutical preparation connoted fair.

[0016] The pharmaceutical preparation of this invention can be continuously prescribed for the patient by prescribing about 1-2000micro g/kg of days, and the amount used as about 10-1000microg/kg for the patient preferably as APC and/or an amount of PC proteins, or enclosing with embedding pharmaceutical preparation, an osmotic-pressure pump, etc., and detaining in a living body by approaches, such as an intravenous injection, subcutaneous injection, an intramuscular injection, intraarticular injection, and internal use.

[0017] (Example of an experiment) Although the example of an experiment explains effectiveness concretely in order to explain this invention below at a detail, this invention is not limited at all by these.

[0018] (The example 1 of an experiment: Effectiveness over the osteoclasis by the Homo sapiens origin APC) Beating of the mouse tibia and femur which removed soft tissue was carried out in the alpha-MEM culture medium, and after agitating by the touch mixer further, the supernatant liquid fraction put and obtained was used as all osteocytes containing an osteoclast. Next, according to approach [bone and mineral (Bone and Mineral) of TAKADA wyes (Takada Y.), 17,347-359, and 1992], the inhibition effectiveness of a tested drug over the resorption cavity formation by the osteoclast on an ivory intercept was investigated. Ivory was prepared to the circular ivory piece with about 150 micrometers [in thickness], and a diameter of 6mm, and it pre incubated in 96 hole plate containing the alpha-MEM culture medium which contains fetal calf serum 5%. After adding all the osteocytes that contain an osteoclast in ivory Kataue and incubating at 37 degrees C for 2 hours, the non-adherent cell was removed, and the alpha-MEM culture medium containing a tested drug was added, and it cultivated for five days. In addition, culture media were exchanged on the 3rd day of culture initiation. The cell which added 0.25M aqueous ammonia on the 5th day of culture, and adhered to ivory Kataue by sonication after suspending a reaction was removed. After dyeing the area of the formed resorption cavity by the acid hematoxylin, it was measured under the microscope. The effectiveness of a tested drug was computed as a rate of inhibition from the area of the resorption cavity of tested drug a non-added group, and a tested drug group. A result is shown in drawing 1 and drawing 2. APC and elcatonin controlled ivory Kataue's resorption cavity formation on the dosage dependence target so that clearly from drawing 1 . This ivory Kataue's resorption cavity formation is the index of osteoclasis, and is controlled by the drugs used for absorptivity bone

diseases, such as elcatonin. Therefore, it is thought that APC is desirable drugs which have prevention / therapy operation of an absorptivity bone disease.

[0019]

[Example] Although an example is given to below and this invention is explained more concretely, this invention is not limited to these.

[0020] (Example 1)

APC 10mg purified gelatin 20mg sodium phosphate 34.8mg sodium chloride 81.8mg mannitol It poured distributively to the dissolution at 10ml of distilled water for injection, after carrying out sterile filtration, the 1.0ml of the 25mg above-mentioned components was poured distributively to each sterile vial, it freeze-dried and the pharmaceutical preparation for injection was adjusted.

[0021] (Example 2)

APC 50mg albumin 20mg sodium phosphate 34.8mg sodium chloride 81.8mg mannitol It poured distributively to the dissolution at 10ml of distilled water for injection, after carrying out sterile filtration, the 1.0ml of the 25mg above-mentioned components was poured distributively to each sterile vial, it freeze-dried and the pharmaceutical preparation for injection was adjusted.

[0022]

[Effect of the Invention] Prevention / therapy agent which makes APC of this invention and/or PC an active principle has osteoclasis depressant action. Therefore, it can use as prevention / therapy agent of absorptivity bone diseases, such as osteopenia by the disease for which various osteoclasis is accelerating, for example, osteoporosis, the malignant hypercalcemia, the Paget's disease of bone, chronic articular rheumatism, etc., and an osteoclasis inhibitor as the acceleration of recovery after fracture, or a reagent for research. Moreover, it is expected compared with the existing medicine that there are few side effects, and APC of this invention and/or PC can serve as a safe remedy in which prolonged administration is possible. Furthermore, since there are few side effects which accompany the therapy by heparin or low molecular weight heparin, such as a bleeding tendency and bone quantity reduction, safety is high [PC] also in case APC of this invention and/or PC are used as an anticoagulant or an agent for dialysis.

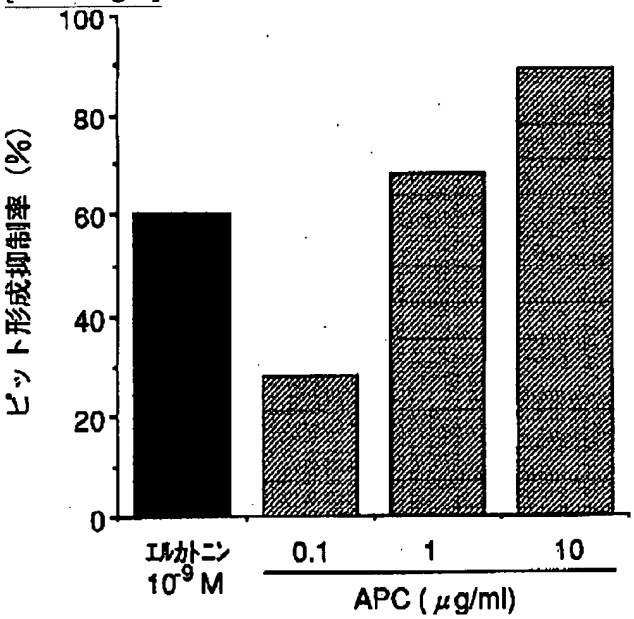
[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

[Drawing 1]



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